J Physiol (2003), **549.3**, *pp.* 937–944

DOI: 10.1113/jphysiol.2003.040170
www.jphysiol.org

Journal of Physiology

The effect of dehydroepiandrosterone on coronary blood flow in prepubertal anaesthetized pigs

C. Molinari, A. Battaglia, E. Grossini, D. A. S. G. Mary, C. Vassanelli and G. Vacca

Laboratorio di Fisiologia, Dipartimento di Scienze Mediche, Facoltà di Medicina e Chirurgia, Università del Piemonte Orientale 'A. Avogadro', Novara, Italy

Extensive research suspecting an association between plasma levels of dehydroepiandrosterone and the risk of coronary heart disease has not been conclusive. The present study was designed to investigate the effect of dehydroepiandrosterone on the coronary circulation and to determine the mechanisms involved. In prepubertal pigs of both sexes anaesthetized with sodium pentobarbitone, changes in left circumflex or anterior descending coronary flow caused by intravenous infusion of dehydroepiandrosterone were assessed using an electromagnetic flowmeter. Changes in heart rate and arterial pressure were prevented by atrial pacing and by connecting the arterial system to a pressurized reservoir containing Ringer solution. In 20 pigs, infusion of 1 mg h^{-1} of dehydroepiandrosterone caused a decrease in coronary flow without affecting left ventricular dP/dt_{max} (rate of change of left ventricular systolic pressure) and filling pressures of the heart. In a further eight pigs, a dose–response curve was obtained by graded increases in the infused dose of hormone between 0.03 and 4 mg h^{-1}. The mechanisms of the above response were studied in the 20 pigs by repeating the experiment after haemodynamic variables had returned to the control values observed before infusion. Blockade of muscarinic cholinoceptors with intravenous atropine (five pigs) and of α-adrenoceptors with intravenous phentolamine (five pigs) did not affect the dehydroepiandrosterone-induced coronary vasoconstriction. This response was abolished by blockade of β-adrenoceptors with intravenous propranolol (five pigs) and of coronary nitric oxide synthase with intracoronary injection of N^{ω}-nitro-L-arginine methyl ester (five pigs) even after reversing the increase in arterial pressure and coronary vascular resistance caused by the two blocking agents with intravenous infusion of papaverine. The present study showed that intravenous infusion of dehydroepiandrosterone primarily caused coronary vasoconstriction. The mechanisms of this response were shown to involve the inhibition of a vasodilatory β-adrenergic receptor-mediated effect related to the release of nitric oxide.

(Received 24 January 2003; accepted after revision 25 March 2003; first published online 17 April 2003)
Corresponding author C. Molinari: Facoltà di Medicina e Chirurgia, Via Solaroli 17, I-28100 Novara, Italy.
Email: molinari@med.unipmn.it

There is virtually no information on the effect of dehydroepiandrosterone, a principal C-19 adrenal steroid of young adults, or its sulfated metabolite on the coronary circulation. The plasma level of this hormone significantly increases following puberty, but declines with advancing age (Parker, 1999). The acute effect of dehydroepiandrosterone on other blood vessels comprised a dose-dependent relaxation of isolated helical strips of rat tail artery that were precontracted by modulation of the intracellular calcium metabolism (Barbagallo *et al.* 1995). In humans an association between plasma levels of dehydroepiandrosterone or its sulfated metabolite and the risk of coronary heart disease has been suspected, though extensive research has not been conclusive (Barrett-Connor *et al.* 1986; Contoreggi *et al.* 1990; LaCroix *et al.* 1992; Mitchell *et al.* 1994; Hautanen *et al.* 1994; Newcomer *et al.* 1994; Herrington, 1995; Barrett-Connor & Goodman-Gruen, 1995*a*,*b*; Johannes *et al.* 1999). Also, it has not been possible to consistently demonstrate an anti-atherogenic effect in humans or experimental animals (LaCroix *et al.* 1992; Nestler *et al.* 1992; Herrington, 1995; Alexandersen *et al.* 1996; Khaw, 1996; Porsova-Dutoit *et al.* 2000).

The present work was therefore designed to study the primary *in vivo* effects of acute administration of dehydroepiandrosterone on coronary blood flow in prepubertal pigs of both sexes and to determine the mechanisms involved. We infused the hormone intravenously whilst preventing changes in heart rate and arterial blood pressure to avoid secondary interference by reflex, local metabolic and physical effects. In addition, a dose–response study was also performed.

METHODS

The experiments were carried out in 28 prepubertal pigs, weighing 68–76 kg, supplied by an accredited dealer (Azienda Cornelia srl, San Pietro Mosezzo, Novara, Italy). The age of the pigs was less than 5 months and 14 of them were male. The animals were fasted overnight and were anaesthetized with intramuscular ketamine (20 mg kg^{-1}; Parke-Davis, Milan, Italy) followed after about 15 min by intravenous sodium pentobarbitone (15 mg kg^{-1}; Siegfried, Zofingen, Switzerland), and artificially ventilated with oxygen-enriched air using a respiratory pump (Harvard 613; Harvard Apparatus, South Natick, MA, USA). Anaesthesia was maintained throughout the experiments by continuous I.V. infusion of sodium pentobarbitone (7 mg kg^{-1} h^{-1}) and assessed as previously reported (Linden & Mary, 1983) from responses of the animals to somatic stimuli. The experiments were carried out in accordance with national guidelines (DLGS 27/01/1992, no. 116).

Pressures in the ascending aorta and in the right atrium were recorded via catheters connected to pressure transducers (Statham P23 XL; Gould, Valley View, OH, USA) inserted into the right femoral artery and the right external jugular vein, respectively. Through left-sided thoracotomy, an electromagnetic flowmeter probe (model BL 613; Biotronex Laboratory, Inc., Chester, MD, USA) was positioned around the proximal part of the left circumflex or the anterior descending coronary artery to record coronary blood flow. A plastic snare distal to the probe was used for zero blood flow assessment. Each probe was calibrated *in vitro* at the end of each experiment.

Left ventricular pressure was measured with a catheter connected to a pressure transducer (Statham P23 XL; Gould) inserted through the left atrium. To pace the heart, electrodes were sewn on the left atrial appendage and connected to a stimulator (model S8800; Grass Instruments, Quincy, MA, USA) which delivered pulses of 3–5 V with 2 ms duration at the required frequency. Arterial blood samples were used to measure pH, arterial partial pressures of oxygen and carbon dioxide (P_{O_2} and P_{CO_2}) (with a gas analyser; IL 1304; Instrumentation Laboratory, Lexington, MA, USA) and the haematocrit. Normal values of pH, P_{O_2} and P_{CO_2} of 7.42 ± 0.02, 84.3 ± 3.9 mmHg and 39.8 ± 0.7 mmHg, respectively, have been reported in prepubertal pigs (Houpt, 1986). In the present study, the animals were artificially ventilated with oxygen-enriched air and values of pH and P_{CO_2} were maintained within normal limits during the experiments by the intravenous infusion of a solution of 2.8 % sodium bicarbonate and by adjusting the respiratory stroke volume, when necessary (Linden & Mary, 1983).

To prevent changes in arterial blood pressure during the experiments, a cannula was introduced into the abdominal aorta through the left femoral artery and connected to a reservoir containing Ringer solution (SIFRA – Società Italiana Farmaceutici Ravizza, Verona, Italy) kept at 38 °C. The reservoir was pressurized using compressed air, which was controlled with a Starling resistance, and pressure within the reservoir was measured by a mercury manometer. In anaesthetized pigs, this method has been shown to allow the arterial blood pressure to be maintained at steady levels without significant changes in filling pressures of the heart or the haematocrit (e.g. Vacca *et al.* 1999; Molinari *et al.* 2002*b*). Coagulation of the blood was avoided by the intravenous injection of heparin (Parke Davis; initial doses of 500 i.u. kg^{-1}, and subsequent doses of 50 i.u. kg^{-1} every 30 min). The rectal temperature of the pigs was monitored and kept between 38 and 40 °C using an electric pad.

Mean and phasic aortic blood pressure, mean right atrial pressure, left ventricular pressure, and mean and phasic coronary blood flow were monitored and recorded together with heart rate and the maximum rate of change of left ventricular systolic pressure (dP/dt_{max}) by using an electrostatic strip-chart recorder (Gould ES 2000; Gould). The heart rate was obtained from the electrocardiogram with a ratemeter (ECG/Biotach amplifier, model 13-4615-65 A; Gould). The frequency response of the differentiator used to obtain left ventricular dP/dt_{max} was found to be flat (± 5 %) up to 150 Hz.

To calculate coronary vascular resistance, the difference between mean aortic blood pressure and mean left ventricular pressure during diastole was considered as the coronary pressure gradient. Coronary vascular resistance was calculated as the ratio between this pressure gradient and mean diastolic coronary blood flow during the steady state. The diastolic period of measurement was defined as starting when ventricular pressure reached its minimum value after systole and ended when it increased at the end of diastole.

At the end of the experiment, each animal was killed by an intravenous injection of 90 mg kg^{-1} sodium pentobarbitone.

Experimental protocol

The experiments were begun after at least 30 min of steady-state conditions with respect to measured haemodynamic variables. In the 28 pigs, to avoid the interference of any possible changes in heart rate and arterial blood pressure during the experiments, the heart was paced to a frequency higher, by 20 beats min^{-1}, than that observed during the steady state and the arterial system connected to the pressurized reservoir. After at least 10 min of steady-state conditions, the experiments were carried out by intravenously infusing 1 mg of dehydroepiandrosterone (Sigma) dissolved in saline, or saline only, in a random order. The infusions were completed in a period of 1 h by using an infusion pump (Model 22; Harvard Apparatus) working at constant rate of 1 ml min^{-1}. After the infusion was stopped, observations were continued for 30 min. The dose of dehydroepiandrosterone used corresponded to that given to elderly subjects to restore their circulating levels to those of young adults (Legrain *et al.* 2000).

Recordings taken for 10 min during the steady state before infusion of dehydroepiandrosterone were used as control. Measurements of haemodynamic variables were obtained during the last 10 min of infusion in the steady state and compared with control values. The effect of infusion of 1 mg h^{-1} of dehydroepiandrosterone on coronary blood flow and the mechanisms involved were studied in 20 pigs. In the remaining eight pigs, a dose–response study was carried out by gradually increasing the infused dose of dehydroepiandrosterone in 12 steps from a minimum value of 0.03 mg h^{-1} to a maximum value of 4 mg h^{-1}. Each dose was infused for 20 min, the smallest of which was estimated to achieve the low serum levels found in adult women (Parker, 1999). The resulting changes in coronary blood flow were compared with control values obtained before starting the infusion.

The mechanisms of the response of coronary blood flow to the infusion of dehydroepiandrosterone were studied in the 20 pigs by repeating the experiment after haemodynamic variables had returned to control levels. In five pigs, dehydroepiandrosterone was administered after blockade of muscarinic cholinoceptors with intravenous administration of atropine (0.5 mg kg^{-1}; Sigma), in five pigs after blockade of α-adrenoceptors with intravenous administration of phentolamine (1 mg kg^{-1}; Ciba Geigy, Varese,

Table 1. Changes in haemodynamic variables caused by intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in 20 pigs

Data	Control	Test	Change
HR (beats min^{-1})	116.9 ± 8.3 (98 to 132)	116.9 ± 8.4 (98 to 132)	0.03 ± 0.13 (−0.1 to 0.4)
ABP (mmHg)	94.8 ± 7.7 (75 to 107)	95 ± 7.5 (76 to 107)	0.2 ± 0.8 (−2 to 2)
dP/dt_{max} (mmHg s^{-1})	2402 ± 247 (2003 to 3023)	2403 ± 247 (2015 to 3023)	1 ± 8 (−13 to 21)
RAP (mmHg)	3.2 ± 0.5 (2.4 to 4.1)	3.2 ± 0.5 (2.4 to 4.1)	0.02 ± 0.09 (−0.1 to 0.3)
LVEDP (mmHg)	5.4 ± 0.8 (4.3 to 7.5)	5.4 ± 0.8 (4.3 to 7.5)	0.03 ± 0.08 (−0.1 to 0.2)
CBF (ml min^{-1})	61.5 ± 8.4 (48.5 to 80.3)	49.5 ± 5.9 (38.4 to 62.3)	−11.9 ± 5 (−28 to −7.3) *

Data are means ± S.D. (range). HR, heart rate; ABP, mean aortic blood pressure; dP/dt_{max}, maximum rate of change of left ventricular systolic pressure; RAP, mean right atrial pressure; LVEDP, left ventricular end-diastolic pressure; CBF, mean coronary blood flow. * $P < 0.0005$ *vs.* control.

Italy), in five pigs after blockade of β-adrenoceptors with intravenous administration of propranolol (0.5 mg kg^{-1}; Sigma) and in the remaining five pigs after blockade of coronary nitric oxide synthase with the intracoronary administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 mg; Sigma). L-NAME was injected into the coronary artery by using a catheter connected to a butterfly needle inserted into the artery. In two of the propranolol-treated pigs and in two of the L-NAME-treated pigs, dehydroepiandrosterone was infused when a steady state had been attained during a continuous intravenous infusion of papaverine (3.5–4.5 mg kg^{-1} h^{-1}; Sigma) to reverse the increase in arterial blood pressure and coronary vascular resistance caused by the blocking agents. All the drugs were administered in the absence of pacing of the heart and without controlling arterial blood pressure. In all subsequent experiments, changes in heart rate and arterial blood pressure were prevented.

Student's paired *t* test was used to examine changes in measured variables caused by dehydroepiandrosterone infusion. A value of $P < 0.05$ was considered statistically significant. Group data are presented as means ± S.D. (range).

RESULTS

In all pigs, recordings commenced approximately 5 h after the induction of anaesthesia. The mean pH, P_{O_2} and P_{CO_2} of arterial blood were 7.39 ± 0.01 (7.37–7.42), 123 ± 11 (106–142) mmHg and 39.3 ± 1 (38–42) mmHg and the haematocrit was 37.8 ± 1.7 (34–40) %.

Effect of infusion of dehydroepiandrosterone

In the 20 pigs, infusion of the vehicle (60 ml of saline) did not cause changes in the control values of measured haemodynamic variables. Group values of data and individual changes in mean coronary blood flow caused by infusion of dehydroepiandrosterone are shown in Table 1 and Fig. 1, respectively. In each pig, infusion of dehydroepiandrosterone caused a decrease in mean coronary blood flow. Group decrease in this flow amounted to 19.1 ± 5.6 (13–35.8) % of the control values and corresponded to an increase in coronary vascular resistance of 22.1 ± 7.8 (10.9–45.1) % from a control value of 1.13 ± 0.17 (0.79–1.49) mmHg ml^{-1} min^{-1}. Changes in left ventricular dP/dt_{max}, mean right atrial pressure and left ventricular end-diastolic pressure were not significant (Table 1). An example of the above response is shown in Fig. 2. In the 20 pigs, the coronary effect of dehydroepiandrosterone began within about 3 min after starting the infusion and reached a steady state in about 5 min. Coronary blood flow returned to control values within 5 min after the end of the infusion. These results indicate that infusion of dehydroepiandrosterone causes coronary vasoconstriction.

Dose–response study

In the eight pigs, control value of mean coronary blood flow was 55.9 ± 6.8 (44.3–66.2) ml min^{-1}. The results obtained by gradually increasing the infused dose of dehydroepiandrosterone are shown in Fig. 3. The threshold dose of the hormone was found to be between 0.05 and 0.1 mg h^{-1}. Maximal coronary effect was observed at a dose of 2.5 mg h^{-1}.

Mechanisms of the response

In the 20 pigs, the infusion of dehydroepiandrosterone was repeated after blockade of muscarinic cholinoceptors and adrenoceptors or after blockade of nitric oxide synthase. The effects of the administration of the blocking agents on

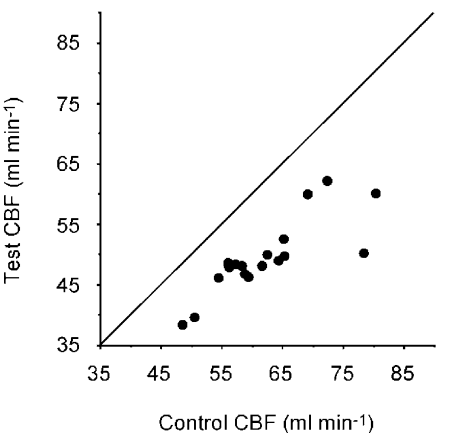

Figure 1. The response of mean coronary blood flow (CBF) to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in 20 pigs

The values of CBF obtained during test period of measurement are plotted on the ordinate against the corresponding control values before infusion on the abscissa. The continuous line is the line of equality.

Table 2. The effects of blockade of cholinoceptors, adrenoceptors and coronary nitric oxide synthase on haemodynamic variables in 20 pigs

	HR (beats min^{-1})	ABP (mmHg)	dP/dt_{max} (mmHg s^{-1})	CBF (ml min^{-1})
Atropine (n = 5)				
Control	96.6 ± 7 (85 to 102)	93.8 ± 8 (83 to 103)	2428 ± 340 (2014 to 2892)	53.1 ± 7.7 (42.8 to 62.2)
Change	10.4 ± 2.1 (8 to 13) *	0.8 ± 3.1 (−3 to 4)	72 ± 60 (−12 to 151)†	0.5 ± 2.2 (−2.8 to 2.7)
Phentolamine (n = 5)				
Control	101 ± 8.2 (90 to 112)	91 ± 6 (82 to 98)	2187 ± 240 (1915 to 2415)	51.1 ± 3.5 (47.2 to 56.1)
Change	14.8 ± 4.8 (8 to 21) §	−16.4 ± 2.5 (−20 to −14) *	7 ± 70 (−84 to 73)	−3.8 ± 5.3(−9.4 to 4.3)
Propranolol (n = 5)				
Control	98.4 ± 8.6 (87 to 110)	87 ± 10.5 (72 to 100)	2343 ± 130 (2200 to 2503)	54.2 ± 12 (41.7 to 68.3)
Change	−24 ± 6.3 (−31 to −15) §	8 ± 2.1 (6 to 11) §	−258 ± 56 (−323 to −192) *	−9.4 ± 2.7 (−13.6 to −6.6) *
L-NAME (n = 5)				
Control	91.4 ± 8.8 (78 to 101)	97.2 ± 4.1 (91 to 100)	2299 ± 186 (2100 to 2502)	51.6 ± 3.3 (47.6 to 55.3)
Change	−5.4 ± 2.3 (−9 to −3) ‡	15.8 ± 5.7 (11 to 24) §	84 ± 39 (54 to 151) ‡	−2.6 ± 3.3 (−6.4 to 2.3)

Data are means ± S.D. (range). HR, heart rate; ABP, mean aortic blood pressure; dP/dt_{max}, maximum rate of change of left ventricular systolic pressure; CBF, mean coronary blood flow. † $P < 0.05$, ‡ $P < 0.005$, § $P < 0.0025$, * $P < 0.0005$ *vs.* control.

control values of heart rate, mean aortic blood pressure, left ventricular dP/dt_{max} and mean coronary blood flow are shown in Table 2. In two of the propranolol-treated pigs and in two of the L-NAME-treated pigs, infusion of papaverine decreased mean aortic blood pressure by 10.5 ± 2.7 (7–13) mmHg ($P < 0.0025$) to the same levels observed before giving the blocking agents, and increased mean coronary blood flow by 7.2 ± 2.2 (4.5–9.8) ml min^{-1} ($P < 0.0025$), with a reduction of coronary vascular resistance of 0.33 ± 0.09 (0.24–0.46) mmHg ml^{-1} min^{-1} ($P < 0.005$). In the same four pigs, the increase in coronary vascular resistance caused by propranolol and L-NAME alone was 0.29 ± 0.08 (0.20–0.40) mmHg ml^{-1} min^{-1} ($P < 0.005$). The above effects of papaverine were accompanied by an increase in heart rate of 5.3 ± 1.3 (4–7) beats min^{-1} ($P < 0.0025$) and a decrease in left

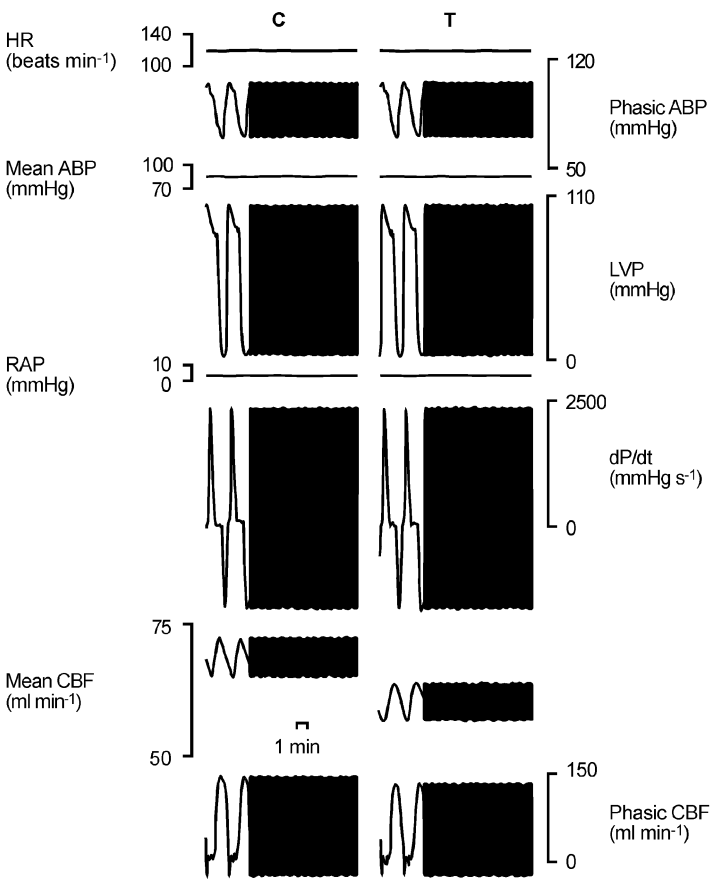

Figure 2. Example of experimental recordings showing the haemodynamic effects of the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone in one pig

C, control before infusion; T, test. From top to bottom: heart rate (HR), phasic and mean aortic blood pressure (ABP), left ventricular pressure (LVP), mean right atrial pressure (RAP), left ventricular dP/dt_{max} (dP/dt), mean and phasic coronary blood flow (CBF).

ventricular dP/dt_{max} of 63 ± 29 (34–91) mmHg s^{-1} ($P < 0.001$).

Experiments after blockade of muscarinic cholinoceptors. Blockade of muscarinic cholinoceptors did not affect the dehydroepiandrosterone-induced decrease of coronary blood flow (Fig. 4). Group decrease in this flow was 8.4 ± 1 (7–9.8 ml min^{-1}, $P < 0.0005$) from a control value of 57.6 ± 9.1 (44.2–66.1) ml min^{-1}. During these experiments, changes in left ventricular dP/dt_{max}, mean right atrial pressure and left ventricular end-diastolic pressure were not significant (at least $P > 0.25$). In the same five pigs, the decrease in mean coronary blood flow obtained with infusion of dehydroepiandrosterone before blockade of muscarinic cholinoceptors was 9.1 ± 1.1 (8.7–10.3) ml min^{-1} ($P < 0.0005$) from control values of 61.8 ± 9 (50.4–72.3) ml min^{-1}. The difference between the two responses before and after blockade was not significant ($P < 0.15$).

Experiments after blockade of α-adrenoceptors. Blockade of α-adrenoceptors did not affect the dehydroepiandrosterone-induced decrease of coronary blood flow (Fig. 4). Group decrease in this flow was 11.7 ± 2.5 (8.7–15) ml min^{-1} ($P < 0.0005$) from a control value of 54.2 ± 9.2 (43.5–68.9) ml min^{-1}. During these experiments, changes in left ventricular dP/dt_{max}, mean right atrial pressure and left ventricular end-diastolic pressure were not significant (at least $P > 0.20$). In the same five pigs, the decrease in mean coronary blood flow obtained with infusion of dehydroepiandrosterone before blockade of α-adrenoceptors was 11.8 ± 2.8 (8.1–15.4) ml min^{-1} ($P < 0.0005$) from control values of 59.9 ± 4.2 (54.3–65.2) ml min^{-1}. The difference between the two responses before and after blockade was not significant ($P > 0.40$).

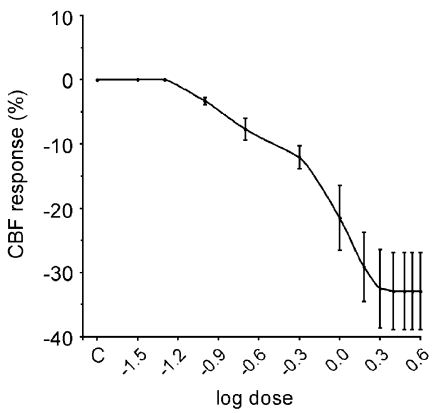

Figure 3. Response of mean CBF to graded increases of the infused dose of dehydroepiandrosterone between 0.03 and 4 mg h^{-1} in eight pigs

The means of percentage changes in CBF obtained during the test period of measurement are plotted against the logarithm of the doses. C, control value before infusions. The bars indicate S.D.

Experiments after blockade of β-adrenoceptors. Blockade of β-adrenoceptors completely prevented the dehydroepiandrosterone-induced decrease of coronary blood flow (Fig. 4). During the test period of measurement, changes in this flow were small and insignificant, amounting to −0.2 ± 0.7 (−1.4 to 0.5) ml min^{-1} ($P > 0.45$) from a control value of 60.5 ± 10.9 (48.2–72.4) ml min^{-1}. During these experiments, changes in left ventricular dP/dt_{max}, mean right atrial pressure and left ventricular end-diastolic pressure were not significant (at least $P > 0.15$). In the same five pigs, the decrease in mean coronary blood flow obtained with infusion of dehydroepiandrosterone before blockade of β-adrenoceptors was 14.8 ± 9 (7.5–28) ml min^{-1} ($P < 0.0125$) from control values of 63.9 ± 14.5 (48.5–80.3) ml min^{-1}. These results indicate that the mechanisms of the observed coronary response to dehydroepiandrosterone involve β-adrenoceptors.

Experiments after blockade of coronary nitric oxide synthase. Blockade of coronary nitric oxide synthase completely prevented the decrease in coronary blood flow to the infusion of dehydroepiandrosterone (Fig. 4). During infusion of the hormone, this flow changed by only −0.1 ± 0.6 (−0.9 to 0.5) ml min^{-1} ($P > 0.35$) from control values of 58.9 ± 4.6 (52.8–64) ml min^{-1}. During these experiments, changes in left ventricular dP/dt_{max}, mean right atrial pressure and left ventricular end-diastolic pressure were not significant (at least $P > 0.25$). In the same five pigs, the decrease in mean coronary blood flow obtained with infusion of dehydroepiandrosterone before blockade of nitric oxide synthase was of 11.1 ± 3.1

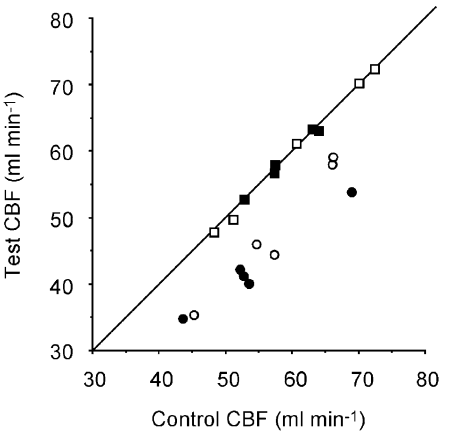

Figure 4. The response of mean CBF to the intravenous infusion of 1 mg h^{-1} of dehydroepiandrosterone after blockade of muscarinic cholinoceptors, adrenoceptors and coronary nitric oxide synthase

The values of CBF obtained in each animal during the test period of measurement are plotted on the ordinate against control values before infusion on the abscissa. The continuous line is line of equality. ○, after blockade of muscarinic cholinoceptors; ●, after blockade of α-adrenoceptors; □, after blockade of β-adrenoceptors; ■, after blockade of coronary nitric oxide synthase.

(7.3–15.1) ml min^{-1} ($P < 0.0025$) from control values of 60.2 ± 4.2 (48.5–80.3) ml min^{-1}. These results indicate that the β-adrenergic receptor-mediated coronary vasoconstriction elicited by infusion of dehydroepiandrosterone involves the release of nitric oxide.

DISCUSSION

The present investigation has shown for the first time that intravenous infusion of dehydroepiandrosterone caused a decrease in coronary blood flow and coronary vasoconstriction. The mechanisms of this effect were shown to involve β-adrenergic sympathetic effects related to the release of nitric oxide.

The decrease in coronary blood flow observed in response to the infusion of dehydroepiandrosterone can be attributed to a primary effect of the hormone and not to a secondary interference by changes in haemodynamic variables. The heart rate and arterial blood pressure were kept constant and there were no significant changes in filling pressures of the heart and left ventricular dP/dt_{max}. This excluded any interference from reflex, local metabolic and physical effects on the coronary response (Feigl, 1983). In addition, infusion of saline alone at the same rate as that of dehydroepiandrosterone did not reproduce the effect of infused hormone. The ability to augment the decrease of coronary blood flow by increasing the dose of infused dehydroepiandrosterone represents a further confirmation of the direct relationship between the hormone and its coronary response. Therefore, the infusion of dehydroepiandrosterone primarily caused coronary vasoconstriction, since this response did not involve changes in other haemodynamic variables. Although myocardial oxygen consumption was not assessed, our findings make it unlikely that changes in myocardial metabolism were involved in this response of coronary vasoconstriction.

Blockade of muscarinic cholinoceptors and α-adrenoceptors did not affect the coronary vasoconstriction elicited by infusion of dehydroepiandrosterone, a response which was shown to be abolished by blockade of β-adrenoceptors. The dose of atropine used in this study has been previously used in anaesthetized pigs to block muscarinic cholinoceptors (e.g. Vacca *et al.* 1999; Molinari *et al.* 2002*b*). The dose of 1 mg kg^{-1} of phentolamine has been shown in the same experimental model to abolish the reflex coronary vasoconstriction caused by distension of the gallbladder (Vacca *et al.* 1996*a*) and has been previously used to block coronary α-adrenoceptors (e.g. Vacca *et al.* 1999; Molinari *et al.* 2002*b*). The dose of propranolol has been previously used in the same experimental model to block β-adrenoceptors (e.g. Vacca *et al.* 1999; Molinari *et al.* 2002). Similar doses of the blocking agents have been used in anaesthetized pigs by other authors to obtain autonomic blockade (Gregory & Wotton, 1981). This indicates that the mechanisms of dehydroepiandrosterone-induced coronary vasoconstriction involved β-adrenergic sympathetic effects. Although propranolol increased baseline arterial blood pressure and coronary vascular resistance, these effects were not involved in the blockade of the coronary response to dehydroepiandrosterone, since infusion of the hormone did not cause significant changes in coronary blood flow even when these increases were reversed by papaverine. However, the propranolol-induced increases in arterial blood pressure and coronary vascular resistance indicated the presence of tonic vasodilatory effects attributable to β-adrenoceptors. This finding is consistent with previous reports showing a tonic β-adrenergic receptor-mediated vasodilatation in the coronary and in several other peripheral vascular beds in anaesthetized pigs (Vacca *et al.* 1996*b*, 1998). Our findings therefore suggest that dehydroepiandrosterone caused coronary vasoconstriction by blocking such tonic vasodilatory effect.

The present findings also showed that the blocking effect of dehydroepiandrosterone on tonic β-adrenergic receptor-mediated coronary vasodilatation involved the endothelial release of nitric oxide, since the coronary vasoconstriction caused by infusion of the hormone was abolished by blockade of nitric oxide synthase with the intracoronary injection of L-NAME. The dose of 100 mg of the blocking agent used has been previously shown in the same experimental model to cause a reduction of the acetylcholine-induced increase in coronary blood flow (Vacca *et al.* 1996*c*, 1999) which was considered a reliable marker of the inhibition of the release of nitric oxide (Parent *et al.* 1992) and has been previously shown to abolish the coronary vasodilatation caused by progesterone and testosterone in anaesthetized pigs (Molinari *et al.* 2001, 2002*b*). As in the case of propranolol, the L-NAME-induced increases in baseline arterial blood pressure and coronary vascular resistance did not influence our results, since dehydroepiandrosterone did not decrease coronary blood flow when these L-NAME-induced increases were reversed by papaverine. The present findings are consistent with previous evidence showing that the release of nitric oxide from the endothelium can modulate or mediate β-adrenergic effects in the coronary and peripheral vasculature. For instance, in dogs it has been shown that an intact endothelium is required for coronary artery responses to the β-adrenergic agonist isoprenaline (Rubanyi & Vanhoutte, 1985) and for the limb vasodilatory response to adrenaline (Young & Vatner, 1986) and that nitric oxide formation contributes to the dilatation of coronary resistance vessels caused by β_2-adrenergic effects (Parent *et al.* 1993). Also, in rats nitric oxide release modulates β-adrenergic vasodilatory responses of the hindlimb (Di Carlo *et al.* 1995) and in pigs the coronary microvessels may be dilated both by β-adrenergic activation and by

stimulation of nitric oxide release, with only a little effect attributable to α-adrenergic receptor activation (Quillen *et al.* 1992). Finally, it is interesting to note that in the pig heart, nitric oxide synthase has been shown to be present mainly in the endothelium of the coronary vessels (Ursell & Mayes, 1993).

The present findings provide important information about the humoral control of the coronary circulation in anaesthetized pigs. For instance, previous reports on the coronary effects of sex hormones have shown that 17β-oestradiol, progesterone and testosterone caused coronary vasodilatation through mechanisms which involved the endothelial release of nitric oxide (Vacca *et al.* 1999; Molinari *et al.* 2001, 2002*b*). On the other hand, growth hormone has been shown to cause a coronary vasoconstriction through the blockade of a β-adrenoceptor-mediated tonic vasodilatory effect related to the endothelial release of nitric oxide (Vacca *et al.* 1998; Molinari *et al.* 2000). Finally, insulin has been shown to cause coronary vasoconstriction as the net result of a vasoconstriction mediated by sympathetic α-adrenergic effects and a vasodilatation related to the release of nitric oxide (Molinari *et al.* 2002*a*).

It is important to point out that these considerations have been derived from the acute effect of administering the hormone in laboratory conditions, a design that enabled quantifying the responses of coronary blood flow whilst avoiding confounding factors. However, our findings have provided data that may be used to propose a role that may be played by sustained changes in the levels of dehydroepiandrosterone. One proposal is that dehydroepiandrosterone-induced coronary vasoconstriction could represent one hormonal mechanism that might balance the effect of coronary vasodilating sex hormones. Also, plasma dehydroepiandrosterone levels are known to be lower in women than in men and to decrease with age in both sexes (Barrett-Connor & Goodman-Gruen, 1995*b*; Johannes *et al.* 1999). The lower value in women could be argued to act in concert with increased coronary vasodilating female hormones to constitute one mechanism, among others, that might underlie the differences in the incidence and severity of coronary artery disease between men and women (Alexandersen *et al.* 1996). It is also tempting to propose that a decrease of dehydroepiandrosterone with age may help protect the coronary circulation in the presence of normally functional endothelium.

In conclusion, the present study has shown that infusion of dehydroepiandrosterone causes coronary vasoconstriction. The mechanisms of this effect were shown to involve the inhibition of a tonic coronary β-adrenergic receptor-mediated effect related to the release of nitric oxide.

REFERENCES

Alexandersen P, Haarbo J & Christiansen C (1996). The relationship of natural androgens to coronary heart disease in males: a review. *Atherosclerosis* **125**, 1–13.

Barbagallo M, Shan J, Pang PK & Resnick LM (1995). Effects of DHEA sulfate on cellular calcium responsiveness and vascular contractility. *Hypertension* **26**, 1065–1069.

Barrett-Connor E & Goodman-Gruen D (1995*a*). The epidemiology of DHEAS and cardiovascular disease. *Ann N Y Acad Sci* **774**, 259–270.

Barrett-Connor E & Goodman-Gruen D (1995*b*). DHEA sulfate does not predict cardiovascular death in postmenopausal women. *Circulation* **91**, 1757–1760.

Barrett-Connor E, Khaw KT & Yen SS (1986). A prospective study of DHEA sulfate, mortality, and cardiovascular disease. *N Engl J Med* **315**, 1519–1524.

Contoreggi CS, Blackman MR, Andres R, Muller DC, Lakatta EG, Fleg JL & Harman SM (1990). Plasma levels of estradiol, testosterone, and DHEAS do not predict risk of coronary artery disease in men. *J Androl* **11**, 460–470.

Di Carlo SE, Patil RD, Collins HL & Chen CY (1995). Local modulation of adrenergic responses in the hindlimb vasculature of the intact conscious rat. *J Physiol* **485**, 817–825.

Feigl EO (1983). Coronary physiology. *Physiol Rev* **63**, 1–205.

Gregory NG & Wotton SB (1981). Autonomic and non-autonomic control of cardiovascular function in stress-sensitive pigs. *J Vet Pharmacol Ther* **4**, 183–191.

Hautanen A, Manttari M, Manninen V, Tenkanen L, Huttunen JK, Frick MH & Adlercreutz H (1994). Adrenal androgens and testosterone as coronary risk factors in the Helsinki Heart Study. *Atherosclerosis* **105**, 191–200.

Herrington DM (1995). DHEA and coronary atherosclerosis. *Ann N Y Acad Sci* **774**, 271–280.

Houpt TR (1986). The handling of swine in research. In *Swine in Cardiovascular Research*, vol. II, ed. Stanton HC & Mersmann HJ, pp. 49–71. CRC Press, Boca Raton, FL, USA.

Johannes CB, Stellato RK, Feldman HA, Longcope C & McKinlay JB (1999). Relation of dehydroepiandrosterone and DHEA sulfate with cardiovascular disease risk factors in women: longitudinal results from Massachusetts Women's Health Study. *J Clin Epidemiol* **52**, 95–103.

Khaw KT (1996). DHEA, DHEA sulphate and cardiovascular disease. *J Endocrinol* **150**, S149–S153.

LaCroix AZ, Yano K & Reed DM (1992). DHEA sulfate, incidence of myocardial infarction, and extent of atherosclerosis in men. *Circulation* **86**, 1529–1535.

Legrain S, Massien C, Lahlou N, Roger M, Debuire B, Diquet B, Chatellier G, Azizi M, Faucounau V, Porchet H, Forette F & Baulieu E (2000). DHEA replacement administration: pharmacokinetic and pharmacodynamic studies in healthy elderly subjects. *J Clin Endocr Metab* **85**, 3208–3217.

Linden RJ & Mary DASG (1983). The preparation and maintenance of anaesthetized animals for the study of cardiovascular function. In *Life Sciences*, vol. P3/1, *Techniques in Cardiovascular Physiology*, ed. Linden RJ, pp. 1–22. Elsevier Science Publishers, Ireland.

Mitchell LE, Sprecher DC, Borecki IB, Rice T, Loskarzewski PM & Rao DC (1994). Evidence for an association between DHEA sulfate and nonfatal, premature myocardial infarction in males. *Circulation* **89**, 89–93.

Molinari C, Battaglia A, Bona G, Grossini E, Mary DASG & Vacca G (2000). The role of nitric oxide in the coronary vasoconstriction caused by growth hormone in anaesthetized pigs. *Exp Physiol* **85**, 203–208.

Molinari C, Battaglia A, Grossini E, Mary DASG, Bona G, Scott E & Vacca G (2002*a*). Effects of insulin on coronary blood flow in anaesthetized pigs. *J Vasc Res* **39**, 504–513.

Molinari C, Battaglia A, Grossini E, Mary DASG, Stoker JB, Surico N & Vacca G (2001). The effect of progesterone on coronary blood flow in anaesthetized pigs. *Exp Physiol* **86**, 101–108.

Molinari C, Battaglia A, Grossini E, Mary DASG, Vassanelli C & Vacca G (2002*b*). The effect of testosterone on regional blood flow in prepubertal anaesthetized pigs. *J Physiol* **543**, 365–372.

Nestler JE, Clore JN & Blackard WG (1992). DHEA: the 'missing link' between hyperinsulinemia and atherosclerosis? *FASEB J* **6**, 3073–3075.

Newcomer LM, Manson JE, Barbieri RL, Hennekens CH & Stampfer MJ (1994). DHEA sulfate and the risk of myocardial infarction. *Am J Epidemiol* **140**, 870–875.

Parent R, al-Obaidi M & Lavallée M (1993). NO formation contributes to beta-adrenergic dilation of resistance coronary vessels in conscious dogs. *Circ Res* **73**, 241–251.

Parent R, Paré R & Lavallée M (1992). Contribution of NO to dilation of resistance coronary vessels in conscious dogs. *Am J Physiol* **262**, H10–16.

Parker CR (1999). Dehydroepiandrosterone and dehydroepiandrosterone sulfate production in the human adrenal during development and aging. *Steroids* **64**, 640–647.

Porsova-Dutoit I, Sulcova J & Starka L (2000). Do DHEA/DHEAS play a protective role in coronary heart disease? *Physiol Res* **49**, S43–S56.

Quillen J, Selke F, Banitt P & Harrison D (1992). The effect of norepinephrine on the coronary microcirculation. *J Vasc Res* **29**, 2–7.

Rubanyi G & Vanhoutte PM (1985). Endothelium-removal decreases relaxations of canine coronary arteries caused by beta-adrenergic agonists and adenosine. *J Cardiovasc Pharmacol* **7**, 139–144.

Ursell PC & Mayes M (1993). The majority of NOS is vascular and not neural. *Cardiovasc Res* **27**, 1920–1924.

Vacca G, Battaglia A, Chiorboli E, Grossini E, Mary DASG, Molinari C & Bona G (1998). Haemodynamic effects of the intravenous administration of growth hormone in anaesthetized pigs. *Pflugers Arch* **436**, 159–167.

Vacca G, Battaglia A, Grossini E, Mary DASG & Molinari C (1996*a*). Reflex coronary vasoconstriction caused by gallbladder distension in anaesthetized pigs. *Circulation* **94**, 2201–2209.

Vacca G, Battaglia A, Grossini E, Mary DASG, Molinari C & Surico N (1999). The effect of 17β-oestradiol on regional blood flow in anaesthetized pigs. *J Physiol* **514**, 875–884.

Vacca G, Mary DASG, Battaglia A, Grossini E & Molinari C (1996*b*). The effect of distension of the stomach on peripheral blood flow in anaesthetized pigs. *Exp Physiol* **81**, 385–396.

Vacca G, Papillo B, Battaglia A, Grossini E, Mary DASG & Pelosi G (1996*c*). The effects of hypertonic saline solution on coronary blood flow in anaesthetized pigs. *J Physiol* **491**, 843–851.

Young MA & Vatner SF (1986). Enhanced adrenergic constriction of the iliac artery with removal of endothelium in conscious dogs. *Am J Physiol* **250**, H892–897.

Acknowledgements

This research has received generous sponsorship by Università del Piemonte Orientale 'A. Avogadro'. We thank the Azienda Ospedaliera Maggiore della Carità di Novara for its help.